Clinical and Experimental Pharmacology & Physiology (1988) **15**, 457–463

THE EFFECT OF TRIFLUOPERAZINE ON BRONCHIAL RESPONSIVENESS IN ASTHMA

J. J. Bowden, K. M. Latimer, A. J. Crockett and R. E. Ruffin

Department of Medicine, Flinders Medical Centre, Bedford Park, South Australia, Australia

(Received 3 August 1987; revision received 16 December 1987)

SUMMARY

1. The protective effects of oral trifluoperazine (TFP) (7mg) against standardized methacholine and histamine inhalation tests (MIT and HIT) were examined 2 and 22 h post-treatment in eight stable asthmatics using a randomized double-blind protocol.

2. A preliminary study tested whether a preceding MIT influenced the result of a subsequent HIT.

3. The mean baseline forced expiratory volumes in 1 s (FEV_1) were similar prior to placebo and TFP. The mean FEV_1 values 2 h after ingestion of placebo or TFP were not different from the baseline values.

4. TFP did not alter bronchial responsiveness to inhaled methacholine or histamine 2 h postingestion, but at 22 h the PC_{20} methacholine was greater than placebo, while PC_{20} histamine did not change. This change in methacholine responsiveness was not clinically significant.

5. There was a correlation between geometric mean provocative concentration of histamine to cause 20% fall in FEV ($PC_{20}H$) for HIT performed in isolation ('separate day') and for HIT performed after MIT ('same day').

6. The effect of inhaled TFP (10 mg/ml, nebulized for 5 min) was examined single-blind and placebo-controlled in a separate group of six stable asthmatics.

7. Inhaled TFP had a bronchoconstrictor effect in all six asthmatics. The mean fall in FEV_1 was 36.4% after inhaled TFP and 2.1% after saline.

8. In the asthmatics studied, ingested TFP exhibited a weak anticholinergic effect 22 h post-treatment, but a brisk spontaneously self reverting bronchoconstrictor response was invariably seen when TFP was inhaled.

Key words: asthma, bronchial responsiveness, bronchoconstriction, calmodulin, histamine, methacholine, trifluoperazine.

Correspondence: Dr R. E. Ruffin, Department of Medicine, Flinders Medical Centre, Bedford Park, SA 5042, Australia.

INTRODUCTION

Contraction of airways smooth muscle is mediated by intracellular calcium-dependent messenger systems. A current model suggests that a rise in intracellular calcium (Ca^{2+}) leads to activation of a calmodulin complex with activation of the enzyme myosin light chain kinase and myosin phosphorylation. Subsequent cyclic attachment and detachment of myosin 'crossbridges' to actin produces smooth muscle contraction (Coburn 1977; Rodger 1985).

Antagonists that block calcium shifts into or within the smooth muscle cells should reduce contraction. Studies of the effects of a variety of calcium channel antagonists in natural and induced asthma have shown clinically unimportant effects (Barnes 1985). However, currently available calcium antagonists have weak effects on shifts through calcium channels and mobilization of intracellular calcium stores in airway smooth muscle. Antagonism of intracellular mediators of the calcium messenger system may be a more potent method of blocking bronchoconstriction (Middleton 1984; Barnes 1985). Currently, no specific calmodulin blocking drugs are available for clinical or experimental use in humans.

Psychotropic drugs, like trifluoperazine (TFP), can inhibit a variety of calmodulin-dependent enzymes through selective calcium-dependent binding to the protein (Roufogalis 1981; Weiss *et al.* 1982). Trifluoperazine has anticholinergic and weak α-adrenoceptor blocking properties in addition to its ability to antagonize calmodulin (Baldessarini 1985). It was postulated that TFP may have a protective effect against induced bronchoconstriction but we did not expect it to have an effect on airways function in stable asthmatics.

In the present study the effect of ingested TFP (7 mg), given double-blind and placebo-controlled, was compared with inhaled methacholine- (MIT) and histamine- (HIT) induced bronchoconstriction, 2 and 22 h post-treatment, in eight stable asthmatics. A separate study was undertaken to ensure that the sequence of MIT and HIT, on the same day, did not bias the measurement. When the ingested pretreatment produced only a small change in methacholine response and larger doses provoked marked drowsiness, a second study was designed to deliver TFP pretreatment (<0.25 mg delivered to the airways), as an aerosol, using a single-blind placebo-controlled protocol.

METHODS

Ingested TFP study

Eight stable asthmatics (six men), aged 18–52 years (mean 32 years), and with no additional medical illness, were recruited from the Respiratory Outpatient Clinic. All had been on the same treatment for at least 6 weeks and were not using antihistamine, phenothiazines or calcium antagonist drugs. Aerosol bronchodilators were withheld for 6 h, and ingested theophylline for 12 h before each study day, but the use of aerosol beclomethasone dipropionate was not interrupted. The subjects attended at the same time of day for each test, rested in the laboratory for 20 min and then baseline forced expired volume in 1 s (FEV_1) was measured in triplicate using a single breath bellows spirometer (Vitalograph). All baseline FEV_1 values had to be within 10% of the value found on the first day for the study to proceed.

A preliminary study was performed to validate that consecutive MIT and HIT on the same day did not introduce error. MIT and HIT were performed as single tests on separate days in random order and then as consecutive tests in the order MIT and HIT on the same day. MIT and HIT were performed by the standardized method described by Cockcroft (1985). Aerosols were generated from a Wright nebulizer with an airflow of 9 l/min producing an output of 0.15 ml/min. A control

saline inhalation was inhaled for 2 min by tidal mouth breathing and then FEV_1 was measured at 30 and 90 s. Subsequently, doubling concentrations of methacholine or histamine were inhaled for 2 min and FEV_1 measured at 30 and 90 s and then at minute intervals until bronchoconstriction eased. The tests were continued until a greater than 20% fall in FEV_1 was achieved from the lowest satisfactory post-saline FEV_1 measurement. The provocative concentration of aerosol causing a 20% fall in FEV_1 ($PC_{20}M$ for methacholine and $PC_{20}H$ for histamine) was obtained from the log dose–response curve by linear interpolation. In the TFP/placebo study, baseline spirometry was measured, the test drug was ingested and 2 h later FEV_1 was remeasured. A MIT was performed, followed 1 h later by a HIT provided that the FEV_1 had returned to >80% of the pre-MIT value; if required, the HIT was delayed for more than 1 h until the FEV_1 reached that value. Twenty-two hours after the test medication, consecutive MIT and HIT were repeated. One week later, the same protocol was completed after ingestion of the alternate pretreatment. Subjects were questioned about drowsiness 24 h after each drug treatment.

Inhaled TFP study

A different cohort of six stable asthmatics (four men), aged 23–51 years (mean 36 years), underwent a HIT to quantitate non-specific bronchial responsiveness ($PC_{20}H$). On two separate test days, the subjects were randomly assigned aerosol inhalation of TFP or saline, given single-blind. The same medication exclusions used in the earlier study applied. Baseline FEV_1 was measured, then TFP (5 ml, 10 mg/ml, pH = 2.1) or acidified saline (pH = 2.1) was inhaled by tidal breathing for 5 min. Each aerosol was generated by a Wright nebulizer (output 0.15 ml/min) and FEV_1 was measured at 30 s, 1.5, 2.5, 3.5, 5, 7.5, 10, 15, 20, 30, 40, 50 and 60 min after completion of the inhalation.

Each study was approved by the Clinical Investigation Committee of Flinders Medical Centre and the participants gave informed written consent.

Statistical analysis

Student's *t*-tests examined all FEV_1 values at baseline and 2 and 22 h after the ingested pretreatment as well as the respective, logarithmically transformed PC_{20} values. Pearson correlation coefficients were calculated to compare separate and same day $PC_{20}H$ values, and $PC_{20}M$ with $PC_{20}H$ values. The reliability of the inhalation challenges with histamine and methacholine was tested by establishing Pearson correlation coefficients for $PC_{20}M$ and $PC_{20}H$ on placebo Day 1 and 2.

Student's *t*-tests examined the maximal % Δ FEV_1 after inhaled TFP and saline. A P value <0.05 was considered significant (Norusis 1986).

RESULTS

Consecutive MIT, HIT vs separate day MIT, HIT

The baseline FEV_1 values were not different before MIT 3.48 ± 0.91 l and before HIT 3.38 ± 0.72 l for separate day tests ($P>0.25$, *t*-test, d.f. = 7). However, the FEV_1 on the same day test was lower preceding the HIT (3.04 ± 0.64 l) than the MIT (3.40 ± 0.78 l; $P<0.01$, *t*-test, d.f. = 7) (Table 1). The correlation coefficient between separate and same day MIT was $r=0.876$ ($P=0.004$), and there was no difference between the geometric mean PC_{20} values of 0.60 for separate day and 0.50 mg/ml for same day test ($P>0.2$, *t*-test, d.f. = 7). The correlation coefficient between separate and same day HIT was $r=0.87$ ($P=0.005$) and there was no difference between

Table 1. Influence of methacholine challenge on subsequent histamine challenge

Subject no.	Separate test day				Same day test			
	FEV_1 (l)	$PC_{20}M$ (mg/ml)	FEV_1 (l)	$PC_{20}H$ (mg/ml)	FEV_1 (l)	$PC_{20}M$ (mg/ml)	FEV_1 (l)	$PC_{20}H$ (mg/ml)
1	2.62	0.33	2.56	0.51	2.58	0.35	2.25	0.78
2	3.52	0.15	3.53	0.22	3.48	0.18	3.22	0.21
3	3.30	1.33	3.35	1.90	3.35	1.65	3.15	1.36
4	4.00	0.76	3.96	0.54	4.02	0.67	3.25	0.78
5	5.10	1.19	4.45	0.55	4.72	0.56	3.95	0.31
6	4.20	0.50	4.00	0.66	3.88	0.27	3.73	0.61
7	2.50	0.40	2.55	0.69	2.65	0.42	2.25	0.60
8	2.65	1.40	2.70	2.08	2.58	0.86	2.50	1.30
Mean	3.48	0.60	3.38	0.70	3.40	0.50	3.04	0.63
s.d.	0.91	0.34	0.72	0.31	0.78	0.34	0.64	0.28

the geometric mean PC_{20} values of 0.70 mg/ml separate day and 0.63 mg/ml for same day test ($P > 0.4$, t-test, d.f. = 7).

Ingested TFP/placebo study

Baseline FEV_1 values prior to placebo 3.35 ± 0.76 l and TFP 3.32 ± 0.91 l were similar ($P > 0.5$, t-test, d.f. = 7). The mean FEV_1 values 2 h after ingestion of placebo (3.24 ± 0.65 l) and TFP (3.24 ± 0.83 l) were not different from the respective baseline values ($P > 0.15$ for both, t-test, d.f. = 7). Similarly, there was no difference in mean FEV_1 values 22 h after ingestion of placebo (3.33 ± 0.92 l) and TFP (3.35 ± 0.68 l; $P > 0.5$, t-test, d.f. = 7). Thus, TFP had no bronchodilator effect at 2 or 22 h post-ingestion.

The geometric mean $PC_{20}M$ was 0.62 mg/ml at 22 h after TFP ingestion and exceeded both the placebo 22 h value of 0.48 mg/ml ($P < 0.05$, t-test, d.f. = 7) and the 2 h $PC_{20}M$ after TFP, 0.42 mg/ml ($P < 0.02$, t-test, d.f. = 7) (Table 2). There was no difference between the geometric mean $PC_{20}H$ values at 3 (Day 1) and 23 (Day 2) h after TFP (0.59 and 0.52 mg/ml respectively), compared with placebo (0.52 and 0.56 mg/ml; $P > 0.25$, > 0.5, t-test, d.f. = 7; Table 3).

Table 2. Effect of ingested TFP and placebo on methacholine inhalation challenge

Subject no.	Placebo				TFP			
	Day 1*		Day 2†		Day 1*		Day 2†	
	FEV_1 (l)	$PC_{20}M$ (mg/ml)	FEV_1 (l)	$PC_{20}M$ (mg/ml)	FEV_1 (l)	$PC_{20}M$ (mg/ml)	FEV_1 (l)	$PC_{20}M$ (mg/ml)
1	2.50	0.31	2.30	0.25	2.25	0.33	2.68	0.57
2	3.20	0.20	3.42	0.19	3.42	0.16	3.35	0.29
3	3.22	0.87	3.35	1.44	3.15	1.14	3.15	2.00
4	3.92	0.92	3.95	0.75	3.62	0.61	3.80	1.04
5	3.93	0.18	4.62	0.43	4.62	0.24	4.38	0.53
6	4.00	0.34	4.08	0.32	3.98	0.35	4.10	0.42
7	2.63	0.42	2.38	0.39	2.62	0.32	2.52	0.43
8	2.55	1.00	2.58	1.05	2.33	1.00	2.85	0.78
Mean	3.24	0.43	3.32	0.48	3.24	0.42	3.35	0.62
s.d.	0.65	0.29	0.91	0.30	0.83	0.29	0.68	0.26

*2 h after and †22 h after ingestion of medication.

Table 3. Effect of ingested TFP and placebo on histamine inhalation challenge after a preceding methacholine inhalation challenge

Subject no.	Placebo				TFP			
	Day 1*		Day 2†		Day 1*		Day 2†	
	FEV_1 (l)	$PC_{20}H$ (mg/ml)	FEV_1 (l)	$PC_{20}H$ (mg/ml)	FEV_1 (l)	$PC_{20}H$ (mg/ml)	FEV_1 (l)	$PC_{20}H$ (mg/ml)
1	2.08	0.37	1.85	0.22	2.22	0.38	2.17	0.36
2	3.10	0.32	3.37	0.31	3.10	0.28	3.00	0.29
3	2.95	0.62	2.93	1.08	2.80	0.72	3.00	1.06
4	3.38	0.53	3.48	0.50	3.22	0.63	3.45	0.55
5	3.55	0.18	4.06	0.31	3.96	0.27	3.60	0.10
6	3.68	0.95	3.78	0.72	3.65	0.83	3.68	0.92
7	2.25	0.43	2.05	1.17	2.17	0.85	2.19	0.83
8	2.45	2.10	2.32	1.10	2.50	1.83	2.45	1.27
Mean	2.93	0.52	2.98	0.56	2.95	0.59	2.94	0.62
s.d.	0.60	0.32	0.82	0.28	0.65	0.28	0.61	0.26

*3 h after and †23 h after ingestion of medication.

The correlation coefficients for the placebo Day 1 and Day 2 $PC_{20}M$ and $PC_{20}H$ values were 0.84 and 0.66 respectively. No patient noted drowsiness after drug administration, and no dystonic reactions were observed.

Inhaled TFP/placebo study

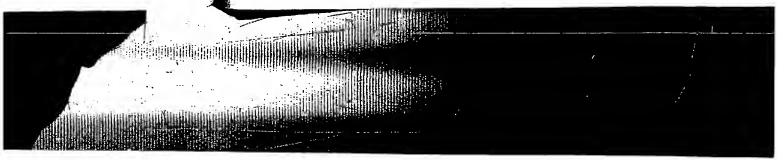
All subjects experienced bronchoconstriction after inhaling TFP (mean maximum FEV_1 fall 36 ± 16.8%) compared with saline (mean maximum FEV_1 fall 2.1 ± 3.4%) ($P<0.005$, *t*-test, d.f. = 5; Table 4). Maximal bronchoconstriction occurred at 4 ± 3 min and by 60 min all subjects had spontaneously returned to >80% of baseline.

DISCUSSION

This study has shown that: a preceding MIT does not significantly affect the result of a subsequent HIT provided that the FEV_1 has recovered to within 80% of the pre-MIT value; oral TFP (7 mg) can protect slightly against a cholinergic stimulus, but in this group of patients has no significant effect on histamine-induced bronchoconstriction; inhaled TFP is a potent bronchoconstrictor

Table 4. Airways effect of inhaled TFP and saline on subjects with a range of histamine responsiveness

Subject no.	$PC_{20}H$ (mg/ml)	Saline		TFP	
		Baseline FEV_1 (l)	Max % FEV_1 fall	Baseline FEV_1 (l)	Max % FEV_1 fall
1	6.0	3.36	3.3	3.28	23.8
2	0.22	2.1	8.5	2.22	67.6
3	1.6	2.32	1	2.34	26.1
4	7.8	3.97	0	3.90	29.8
5	2.4	2.45	0	2.45	43.6
6	5.6	3.3	0	3.25	27.3



through mechanisms as yet unknown; bronchial responsiveness measured as $PC_{20}H$ and $PC_{20}M$ produces similar values (Juniper *et al.* 1978); and both inhalation challenges are reproducible tests (Cockcroft 1985).

Results from previous studies of repeated provocation tests have been conflicting. Ruffin *et al.* (1981) showed that four histamine tests could be repeated with 40 min intervals without tachyphylaxis in 12 moderately severe asthmatics. In contrast, Manning *et al.* (1987) showed that tachyphylaxis can occur with three repeated histamine inhalation tests in a group of mild asthmatics. However, Manning and O'Byrne (1987) have shown that acetylcholine inhalation does not cause tachyphylaxis to subsequent acetylcholine inhalation nor affect subsequent histamine inhalation in mild asthmatics. The present study confirms that a preceding cholinergic inhalation challenge does not affect a subsequent histamine challenge.

No published data are available regarding TFP and airways responsiveness, although tricyclic antidepressants (which share structural similarities with TFP) have been thought to ameliorate asthma (Meares *et al.* 1971). Further, *in vitro* studies have not specifically examined the effect of TFP on airways smooth muscle. Hence, it is difficult to predict the optimal dose of TFP required. The dose of 7 mg used in this study was chosen for two reasons. First, when prescribed as a major tranquilizer, the initial dose in adults is 5 mg, and second, a single dose of 10 mg was associated with excessive drowsiness in one investigator. Facilities for the measurement of blood levels of the drug were not available.

However, the observation of an effect of oral TFP on methacholine challenge but not on histamine challenge, suggests that the dose used was sufficient to achieve a local anticholinergic effect on airways smooth muscle. While this anticholinergic effect is statistically significant, it is small and not clinically important, but it is consistent with the previously observed weak cholinergic blocking effects of antipsychotic agents (Baldessarini 1985). The failure to observe protection against histamine-induced bronchoconstriction in this study may be due to the oral TFP dose being insufficient to block calmodulin or to calmodulin inhibition not being a potent protective mechanism for bronchial smooth muscle contraction. The former possibility was investigated by using the aerosol formulation. Surprisingly, each subject bronchoconstricted with this treatment. The mechanism for this was not pH-dependent (saline control had an equivalent pH of 2.1) and is unlikely to be due to a hyperosmolar challenge as 10 mg/ml TFP in saline solution has an osmolality of 304 mmol/kg.

An *in vitro* study by Paechell and Pearce (1985) has shown that compounds with calmodulin antagonist properties (including TFP) have a dual effect on histamine release by rat peritoneal mast cells. At high concentrations these compounds released histamine by cell autolysis, while at low concentrations they blocked secretion of histamine to immunological and pharmacological challenges. Hence mast cell release of histamine may explain the bronchoconstrictor effect of inhaled TFP. This could then mask any protective calmodulin inhibition. The other alternative is that the lack of protection of oral TFP may indicate that non-calmodulin-mediated pathways are involved in smooth muscle contraction. Rasmussen (1986) suggested that a calmodulin-mediated process is involved in the short phase or initial smooth muscle contraction, while a C kinase protein system mediates sustained contraction. Hence, antagonism of calmodulin alone may be insufficient to block smooth muscle contraction. Other pharmacological properties of TFP, such as α-adrenoreceptor antagonism, cannot be invoked as bronchodilatation was not observed in the present study.

This study does not preclude a role for calmodulin antagonists but does suggest that: the weak anticholinergic effect of low dose ingested TFP is clinically unimportant and systemic side effects prohibit the use of larger doses; inhaled TFP (<0.25 mg) causes bronchoconstriction, but further

ss measured as $PC_{20}H$ and $PC_{20}M$
on challenges are reproducible tests

s have been conflicting. Ruffin *et al.*
d with 40 min intervals without
, Manning *et al.* (1987) showed that
halation tests in a group of mild
n that acetylcholine inhalation does
on nor affect subsequent histamine
t a preceding cholinergic inhalation

/s responsiveness, although tricyclic
P) have been thought to ameliorate
t specifically examined the effect of
t the optimal dose of TFP required.
is. First, when prescribed as a major
single dose of 10 mg was associated
e measurement of blood levels of the

methacholine challenge but not on
ns to achieve a local anticholinergic
effect is statistically significant, it is
with the previously observed weak
arini 1985). The failure to observe
this study may be due to the oral
odulin inhibition not being a potent
tion. The former possibility was
ith subject bronchoconstricted with
(saline control had an equivalent
ects 10 mg/ml TFP in saline solution

and that compounds with calmodulin
stamine release by rat peritoneal mast
stamine by cell autolysis, while at low
mmunological and pharmacological
ith the bronchoconstrictor effect of
in inhibition. The other alternative is
n-calmodulin-mediated pathways are
suggested that a calmodulin-mediated
muscle contraction, while a C kinase
agonism of calmodulin alone may be
macological properties of TFP, such as
hodilatation was not observed in the

gonists but does suggest that the weak
unimportant and systemic side effects
nuses bronchoconstriction, but further

studies are required to assess the possible protective effect of lower doses; and calmodulin antagonists may need to be tested in combination with agents capable of blocking membrane or intracellular calcium-dependent pathways, for clinically useful effects in the asthmatic airways.

ACKNOWLEDGMENTS

The authors acknowledge a grant from Fisons Pty Ltd which was used to support this study. Smith Kline & French donated trifluoperazine HCl substance and analysed the trifluoperazine solution.

REFERENCES

Baldessarini, R.J. (1985) Drugs and the treatment of psychiatric disorders. In: *The Pharmacologic Basis of Therapeutics* (Ed. A.S Gilman, L.B. Goodman, T.W. Rall & F. Murad), Chap. 19, pp. 404–417. Macmillan, New York.

Barnes, P.J. (1985) Clinical studies with calcium antagonists in asthma. *British Journal of Clinical Pharmacology*, 20, S289–298.

Coburn, R.F. (1977) The airway smooth muscle cell. *Federation Proceedings*, 36, 2692–2697.

Cockcroft, D.W. (1985) Measurement of airway responsiveness to inhaled histamine or methacholine: method of continuous aerosol generation and tidal breathing inhalation. In: *Airway Responsiveness: measurement and interpretation*, (Ed. F.E. Hargreave & A.J. Woolcock), pp. 22–28. Astra Pharmaceuticals Canada, Ontario.

Juniper, E.F., Frith, P.A., Dunnett, C., Cockcroft, D.W. & Hargreave, F.E. (1978) Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax*, 33, 705–710.

Manning, P.J., Jackson, P. & O'Byrne, P.M. (1987) Cimetidine prevents histamine tachyphylaxis in asthmatic subjects. *Thorax*, 42, 748.

Manning, P.J. & O'Byrne, P.M. (1987) Histamine stimulation causes tachyphylaxis to both inhaled histamine and acetylcholine in asthmatic subjects. *Thorax*, 42, 712 (abs).

Mearns, R.A., Mills, J.E. & Horvath, T.B. (1971) Amitriptyline and asthma. *Medical Journal of Australia*, 2, 25–28.

Middleton, E. (1984) Airway smooth muscle, asthma and calcium ions. *Journal of Allergy and Clinical Immunology*, 73, 643–650.

Norusis, M.J. (1986) *SPSS/PC+. For the IBM PC/XT/AT*. SPSS, Chicago.

Peachell, T. & Pearce, F.L. (1985) Effect of calmodulin inhibitors on histamine secretion from mast cells. *Agents and Actions*, 16, 43–44.

Rasmussen, H. (1986) The calcium messenger system (Parts 1 and 2). *New England Journal of Medicine*, 314, 1164–1170, 1094–1100.

Rodger, I.W. (1985) Excitation-contraction coupling and uncoupling in airway smooth muscle. *British Journal of Clinical Pharmacology*, 20, S255–266.

Roufogalis, B.D. (1981) Phenothiazine antagonism of calmodulin: a structurally nonspecific interaction. *Biochemical and Biophysical Research Communications*, 98, 607–613.

Ruffin, R.E., Alpers, J.H., Crockett, A.J. & Hamilton, R. (1981) Repeated histamine inhalation tests in asthmatic patients. *Journal of Allergy and Clinical Immunology*, 67, 285–289.

Weiss, B., Prozialeck, W.C. & Wallace, T.L. (1982) Interaction of drugs with calmodulin. Biochemical, Pharmacological and clinical implications. *Biochemical Pharmacology*, 31, 2217–2226.